

LABORATORY EXERCISES FOR

ELEMENTS OF BIOLOGY

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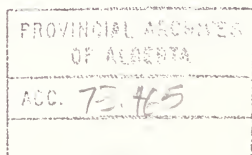
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Laboratory Exercises for

ELEMENTS of BIOLOGY

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Laboratory Exercises for
Elements of Biology

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Preface

This manual is a guide and framework, not a prescription. The first essential quality in a scientist is curiosity: no regulations must be allowed to interfere with the fostering of this quality whenever it shows itself, in student and in teacher alike. The 32 exercises outlined allow time for this in a forty-week teaching year. The second essential in a scientist is integrity: this must be reflected in every observation and especially in every interpretation. Students should learn early to glory in what Huxley defined as the greatest tragedy of science: "the slaying of a beautiful hypothesis by an ugly fact."

This manual has been prepared with the specific aims of drilling the student in the basic procedures which must be followed in making scientific observations and in drawing conclusions from them, and of encouraging student-teacher discussion of biological principles during laboratory periods.

The standard scientific procedure of recording data directly in a permanent laboratory book at the time of observation must be followed throughout this course.

It will be necessary for the student to do some prior reading of the textbook and lecture material in order to perform experiments in biology adequately. Problems which are brought to light by this review can then be discussed with the teacher, who will lead the discussion towards the answers. In addition, questions arising from attempts to interpret the results obtained can be profitably discussed with the teacher. If these procedures are followed, most of the report may be drafted during the laboratory period, thus reducing the total time necessary for the study of the topic to which the experiment relates.

Most of the exercises can be completed in one period of forty-five minutes. Better use can be made of time if longer or double periods are available. Many pairs of experiments are arranged in sequence with this possibility in mind.

Instructions to Students

1. Students are required to provide a separate looseleaf binder for a laboratory exercise book. Both lined and unlined pages are necessary. Teachers may prefer to have students write on one side of the paper only — check with your teacher before writing up your laboratory reports.
2. All laboratory data must be recorded directly in the laboratory notebook as the observations are made.
3. Students are to work in groups of four unless otherwise instructed.
4. After each laboratory period, all used laboratory glassware and apparatus must be thoroughly cleaned and returned to its proper place of storage.
5. Any defective apparatus must be reported to the teacher immediately.
6. All dissection equipment, whether your own or the property of the school, must be carefully cleaned and dried before being put away. The life and efficiency of these instruments is extended by proper care.
7. All plant or animal refuse is to be put into the special containers provided, NOT in the sinks or wastebaskets.

Writing up Laboratory Reports These reports should have a form similar to that of published scientific papers. They should be written in simple, clear, straightforward sentences. Good English is demanded. Experiments must be written up within one week after they are performed.

An outline of what is required follows:

Date _____
Lab. No. _____

Group Members:

1. _____ (recorder)
2. _____
3. _____
4. _____

TITLE A short statement indicating what the experiment is about.

OBJECT State the purpose of the experiment and the biological principle it is designed to teach.

PROCEDURE A brief summary, in general terms, indicating what preparation was used and what was measured. Do not copy out the detailed directions from the manual, but if your procedure differs from that in the manual this difference should be outlined in your report. If the experiment includes several sections use the same main headings and numbering as that used in the manual. For most experiments this section should not exceed a single short paragraph.

RESULTS Here the data are presented as originally recorded, in the form of tables, graphs, or charts. All data must be clearly labelled and must be accompanied by headings.

INTERPRETATION This portion of the report should immediately follow the results and should show evidence of insight and understanding of the topic under study. If the results do not turn out as you expected, account for the discrepancies. This will no doubt require further reading and investigation but the time spent will provide you with a deeper understanding and appreciation of the ways of science.

QUESTIONS Answer all questions which are in the laboratory manual.

REFERENCES It may be necessary for you to refer to the text and reference books to support many of your statements. List the names of the references and give specific page number(s). The following example illustrates one accepted method:

Weisz, Paul B., *Elements of Biology*, McGraw-Hill Company of Canada Limited, Toronto. 1961. Page 117.

Evaluation of Laboratory Work 1. High marks will be given only for reports which follow the prescribed pattern. Brief but complete reports are preferred.

2. Performance in the laboratory will be assessed by tests as well as by means of written reports in the laboratory notebook.

3. The student may include a section on theory, which should follow the object of the experiment. This is an optional aid to study.

You are embarking on a voyage of discovery over parts of the sea of life unknown to you. As you yourself live, go well prepared. Take with you maps (your text and whatever else you can read among the vast literature of biology); take your teacher as a pilot of some experience on this sea; take your curiosity as motive power; and use your honesty as a compass. Bon voyage, safe return, and a fuller life thereafter.

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POISON WARNING

Many substances used in the biological laboratory are poisonous. This applies especially, of course, to materials used to kill and preserve animals. Some materials may be infective.

When in doubt about a substance, treat it as if it were poisonous. Always wash your hands thoroughly after working in the laboratory and before eating or drinking. Attend carefully to any instructions or precautions which may be given on a container.

Composition of Protoplasm - Carbohydrates

OBJECT *to identify monosaccharides, disaccharides, and polysaccharides.*

MATERIALS

Test tubes—11	Iodine potassium iodide solution (I, KI
Test tube rack	(see appendix)
Test tube holder	Distilled H ₂ O
Bunsen burner	10% formaldehyde solution
Medicine dropper	1% starch suspension
Benedict's solution	1% glycogen solution
	1% glucose solution
	1% fructose solution

PROCEDURE

Colour reactions with specific reagents can be used to identify carbohydrates. Monosaccharides, disaccharides, and polysaccharides, each require different testing procedures. Number seven test tubes, and prepare a table as follows in your notebook. Proceed with steps 1 and 2, and then interpret your results.

Tube No.	Step #1. Add 1 ml. of	Step #2. Add 3 ml. Benedict's solution to each. Bring to the boil and record the results.
1	formaldehyde	
2	glycogen	
3	sucrose	
4	maltose	
5	fructose	
6	glucose	
7	distilled H ₂ O	

(1) *Why was the tube of distilled H₂O used?*

Polysaccharides Number four test tubes to be used as indicated in the following table:

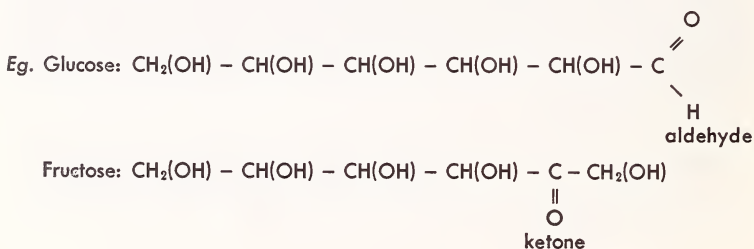
Tube No.	Step #1. Add 2 ml. of	Step #2. Add 2-3 drops of iodine solution and record the results.
1	starch suspension	
2	glycogen solution	
3	glucose solution	
4	distilled water	

(2) *Is the iodine test suitable for distinguishing between starch and glycogen?*

(3) *For what carbohydrates is the iodine test suitable?*

INTERPRETATION

Monosaccharides are simple sugars represented by the general chemical formula $C_6H_{12}O_6$. In protoplasm the only reducing substances of importance are aldehydes and ketones, i.e., compounds containing free aldehyde or ketone groups.



When Benedict's solution is added to these reducing sugars, the cupric ion is reduced to yellowish-orange cuprous oxide (Cu_2O). The colour varies with the amount of reducing substance present. This is because if not all the copper is reduced, the yellow cuprous oxide is seen through a solution which is still blue because of cupric ions; the mixture thus appears greenish. In the sucrose molecule, glucose and fructose are joined by the aldehyde and ketone groups so that these groups are no longer free. The presence of a free ketone or aldehyde group in a sugar makes it a good reducing agent. All of the simple sugars and the common double sugars, except sucrose, contain one or both of these groups. Sucrose is a non-reducing sugar and therefore gives a negative result with Benedict's solution.

Composition of Protoplasm - Proteins

OBJECT to identify proteins.

MATERIALS

#6 test tubes—6	400 ml. beakers—2
Test tube rack	Bunsen burner
HgCl ₂ saturated aqu. sol.	Distilled water
Concentrated NaOH	100 ml. graduate cyl.
0.5% CuSO ₄ • 5H ₂ O solution	Concentrated HCl
Egg—1	

PROCEDURE

Coagulation test Separate the yolk from the white (albumin) of one egg. Put each in a 400 ml. beaker and add 50 ml. of distilled water to each. Stir well. Number four test tubes; put 2 ml. of diluted egg white in each, and add to #1, 2 ml. of HgCl₂, #2, 2 ml. of concentrated HCl, #3, 2 ml. of concentrated NaOH, #4, nothing. Record your results in a table with column headings: *Tube No.*, *Material Added*, *Results*, and line headings: 1, 2, 3, and 4.

Biuret test A combination of protein, concentrated NaOH, and dilute CuSO₄ forms a complex compound called biuret which has a colour varying from pink to purple.

Number two test tubes. Add 2 ml. of yolk solution to one and 2 ml. of albumin solution to the other. To each add 2 ml. of concentrated NaOH. Then add to each tube a few drops of 0.5% CuSO₄ solution. Do not shake or mix. Interpret the results.

Lab. 3

Composition of Protoplasm - Lipids

OBJECT *to identify lipids.*

MATERIALS

#6 test tubes—2

Test tube rack

CCl_4 —3 ml.

Olive oil—1 ml.

Distilled water

Unglazed brown paper

Sudan IV powdered dye

Medicine droppers—2

10 ml. pipette with rubber bulb

Substances which contain a high percentage of fats can easily be identified by the familiar grease marks which they make on unglazed brown paper. However, if the concentration of fat is low, a more sensitive test is required.

PROCEDURE

Grease spot test Number two test tubes, and add 3 ml. of distilled water to each. To tube number one add 1 ml. of CCl_4 and to tube number two add 1 ml. of olive oil. Shake both tubes thoroughly and set aside.

(1) *Does the water mix in either tube?*

To tube #2 add 1 ml. of CCl_4 , shake well, and set aside.

(2) *Is there evidence of separation of contents? If so, of what does each layer consist?*

Use one medicine dropper to transfer a drop of liquid from the top layer of tube #1 to the brown paper. Use another dropper to transfer a drop of liquid from the top layer of tube #2 to another spot on the brown paper. Allow the paper to dry. Observe and record the results.

Sudan IV dye test for lipids The principle of this test is that Sudan IV is insoluble in water but soluble in lipids. Thus, if the dye dissolves even slightly and discolours the liquid, it proves the presence of a lipid.

To 3 ml. of distilled water in a test tube, add a small amount of Sudan IV dye. Shake well and observe. Now add 1 ml. of olive oil to the mixture, shake well, and let stand for 5 minutes. Record the results.

Composition of Protoplasm - Mineral Ions

OBJECT *to identify mineral ions.*

MATERIALS

#6 test tubes—8	CaCl ₂ 10% solution—3 ml.
Test tube racks—2	Oxalic acid 5%
NaCl 10% solution—3 ml.	Potassium ferrocyanide 5%
(NH ₄) ₃ PO ₄ 10% solution—3 ml.	HCl—0.1 N
CuSO ₄ ·5H ₂ O 10% solution—6 ml.	AgNO ₃ 2% solution
Na ₂ CO ₃ 10% solution—3 ml.	BaCl ₂ solution
NaHCO ₃ 10% solution—3 ml.	Dilute HNO ₃
FeCl ₃ 10% solution—3 ml.	

Colour or precipitation tests can be used to identify most of the biologically important inorganic ions. Flame tests can also be used, but the results are often unsatisfactory.

PROCEDURE

Number eight test tubes.

Phosphate ions To tube #1 add 3 ml. of 10% solution of (NH₄)₃PO₄. Add 1 ml. of 2% AgNO₃, and observe results. Add 2 or 3 ml. of dilute HNO₃. Observe and record all results in tabulated form in your notebook.

Carbonate and bicarbonate ions To tube #2 add 3 ml. of Na₂CO₃ solution, and to tube #3 add 3 ml. of NaHCO₃ solution. To each add a few drops of 0.1 N HCl. Observe and record results. Write a balanced chemical equation for each reaction.

Chloride ions To tube #4 add 3 ml. of NaCl solution. Add a few drops of a 2% AgNO₃ solution. Observe and record results. (NOTE: bromides and iodides give similar results).

Calcium ions To tube #5 add 3 ml. of a CaCl₂ solution and a few drops of 5% oxalic acid (H₂C₂O₄). Observe and record results.

Ferric ions To tube #6 add 3 ml. of FeCl₃ solution and a few drops of potassium ferrocyanide (K₄FeCN₆). Observe and record results.

Cupric ions To tube #7 add 3 ml. of a CuSO_4 solution and a few drops of potassium ferrocyanide. Observe and record results.

Sulphate ions To tube #8 add 3 ml. of a CuSO_4 solution and a few drops of BaCl_2 . Observe and record results.

Composition of Protoplasm - Biological Materials

OBJECT *to identify protoplasmic constituents in biological material.*

MATERIALS

One or more of the following:

Milk
Urine
Blood plasma
Potato water
Beef broth

Orange juice or unknown solutions supplied by the teacher (These may contain a single substance or mixtures of several substances, organic, inorganic, or both.)

PROCEDURE

Carry out the tests in Labs 1 to 4 on each substance. Record your results in a table with column headings showing substance tested, and line headings for the tests carried out. A complete list would show tests for: Reducing sugars, Polysaccharides, Proteins, Lipids, Phosphate, Carbonate or bicarbonate, Chloride, Calcium, Iron, Copper, Sulphate.

Lab. 6

Molecular Movement

OBJECT *to observe evidence of diffusion.*

MATERIALS

Powdered carmine dye
Microscope
Microscope slide
Cover slip
Large test tube

Powdered Janus green dye
400 ml. beaker
 NH_4OH
Phenolphthalein
1 sheet filter paper

PROCEDURE

Brownian movement This phenomenon provides visible evidence of molecular motion.

Place a drop of water on a clean microscope slide, and add several grains of powdered carmine dye. Cover and examine under high power of the microscope. While molecules are too small to be seen under the microscope, their impacts will cause the visible dye particles to move. The peculiar vibrating movement of these tiny particles is known as Brownian movement.

- (1) *Why do particles appear to vibrate rather than travel in one direction?*
- (2) *What effect, if any, would increasing the temperature have in this demonstration? Why?*

Diffusion Diffusion may be described as the movement of molecules in response to a difference of concentration. Since the tendency is for a movement from regions of higher concentration to those of lower, diffusion tends to equalize concentration. The process by which water molecules diffuse through a semipermeable membrane into a solution, where the molecules are less concentrated, is called *osmosis*.

Diffusion of a solid in a liquid Place 300 ml. of water in a 400 ml. beaker. Set in a place of uniform temperature. Sprinkle several grains of Janus green powdered dye on the surface of the water. Observe at 10 minute intervals. Record and explain the results.

Diffusion of a gas in a gas Wet some filter paper with phenolphthalein, and press it into the bottom of a long test tube. Invert over an open bottle of NH_4OH . Observe, record, and explain results.

INTERPRETATION

All elements and compounds are made up of molecules. At ordinary temperatures and pressures, the molecules of a gas are extremely far apart in comparison with their sizes. They move in all directions and at high speeds. The molecules constantly collide with one another and with those of any other gas present. This random movement accounts for the fact that the molecules always disperse themselves evenly throughout any containing vessel.

The differences between the solid, liquid, and gaseous states are thought to be due to differences in the energy of the molecules and hence to differences in the inter-molecular spaces. Gases have the greatest inter-molecular spaces, liquids have less, and solids the least.

Osmosis

OBJECT *to study osmosis and its relation to tonicity.*

MATERIALS

Part A

Beaker—100 ml.
Commercial water-glass
(50% solution sodium silicate)—75 ml.
Two or more of:
 $\text{Fe}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ —1 crystal
 FeCl_3 —1 crystal
 $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ —1 crystal
 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ —1 crystal
 $\text{Cr}(\text{NO}_3)_3$ —1 crystal

Part B

KMnO_4
NaOH concentrated
NaCl
250 ml. beakers—3
Phenolphthalein
String—2 feet
Scissors—1 pair
Distilled water—400 ml.
Ethylene glycol—100 ml.
Glycerine—25 ml.
Dialysis tubing—6 inches

PROCEDURE

Part A

Fill a 100 ml. beaker three-fourths full of water-glass solution. Drop in a small crystal of each of the following compounds: ferric nitrate, ferric chloride, ferrous sulphate, copper sulphate, chromic nitrate. Do not agitate or jar the beaker. Observe for five or ten minutes, and record the results in a table.

INTERPRETATION

The salts listed are soluble in water. When placed in a water glass solution, the sodium silicate of the water-glass reacts with the salts to form a membrane layer of the silicate of iron, copper, or chromium between the dissolving salt and the solute. This establishes an osmotic system.

- (1) *What are the solutions within the membranes?*
- (2) *What substance passes through the membranes?*

- (3) *Why does the membrane break and reform?*
- (4) *What term can be used to describe any of these membranes which have formed?*

Part B.

Cut two pieces of dialysis tubing each 3 inches long. Wet the tubing, and open it by rolling it between the fingers. Tightly tie off one end of each tube. Partially fill one tube with glycerine to which a few drops of concentrated NaOH have been added. Tie the remaining end of the tube, but leave space for expansion within the tube. Be sure the sac formed is watertight. Place this tube into a 250 ml. beaker of distilled water to which a few drops of phenolphthalein have been added. Set it aside, and observe at the end of the period and also 24 hours later. Call this tube #1. (Save the expanded tube for a later part of this experiment.)

Half fill the second tube with distilled water. Tie tightly, and drop into a 250 ml. beaker of distilled water in which a few crystals of KMnO_4 have been dissolved. Set aside. Observe at the end of the period and after 24 hours. Rinse the outside of the bag before making observations. This is tube #2.

Place the tube saved from the first part of the experiment in a 250 ml. beaker containing ethylene glycol. This is tube #3. Set aside; observe at the end of the period and 24 hours later. Record all your results in table form. Column headings: *Tube Number*, *Observation at end of period*, *Observation after 24 hours*, line headings: *Tube #1*, *Tube #2*, *Tube #3*.

- (1) *Explain in terms of hypertonicity, isotonicity, and hypotonicity what has happened in each part of this experiment.*
- (2) *Explain diffusion in terms of the kinetic-molecular theory.*

Properties of Protoplasm - Gelation

Protoplasm is a polyphasic dispersion in which each phase has a complex composition. Protoplasm is simultaneously a colloidal and a crystalloidal dispersion — partly a suspension, partly an emulsion, and partly a solution. Sometimes protoplasm is in a sol state, and sometimes it is a gel. This complex structure of living matter is not static but is continually changing, and these changes are an integral part of the vital activities of the living cell.

OBJECT *to study the effects of temperature change, dilution, and ion concentration on gelation and solation.*

MATERIALS

#6 test tubes—7	Asbestos wire gauze
Distilled water	NaCl
Powdered gelatin	CaCl ₂
Bunsen burner	CaSO ₄
Beaker—400 ml.	Grease pencil
Ring stand	

PROCEDURE

Prepare two test tubes of gelatin by dissolving 1 gram of gelatin in 10 ml. of distilled water in each tube. Gentle heating may be required to dissolve the gelatin. Cool thoroughly in a beaker of ice or cold water. When the gelatin has set, heat both tubes in a water bath.

(1) *What happened to the gelatin when cooled and when heated?*

Dilute the solution in one of the tubes by filling it with distilled water. Cool both tubes as before. Explain your results fully by considering the concentrations of colloidal particles in the tubes.

Number three test tubes. Add 1 gram of dry gelatin and 10 ml. of distilled water to each tube. Dissolve gelatin as above and set by cooling as in the previous exercise.

Add 1 gram of solid crystals of the following substances, one type only to each tube: #1 CaSO_4 , #2 NaCl , #3 CaCl_2 . Set these aside; observe them at the end of the lesson period and again after 24 hours.

INTERPRETATION

Protoplasm frequently changes its state by undergoing gelation or solation. Like a gelatin solution, protoplasm may become set at a certain moment into a semi-solid, elastic mass called a *gel*. Later it may revert to a more fluid state called a *sol*. Several factors such as temperature change and concentration of colloidal particles may cause this sol-gel reversal.

To interpret the results of this experiment, it is necessary to examine the ion-electrical properties of the salts used.

(2) *Write the ionic equations for the dissociation of each of the above salts. (Note: not all salts dissociate.)*

Before any colloidal system can attain a gel state, it is essential that all colloidal particles carry the same electrical charge. Thus, any given particle will be repelled by all other particles surrounding it and will be held in a relatively fixed position. However, colloidal particles adsorb to their surfaces ions of opposite charge, if such ions are present in the colloidal dispersion. This adsorption often releases heat because of the electrical exchange taking place.

If the charges on the colloidal particles are neutralized by the adsorbed ions of opposite charge, then the gel will be changed into a sol.

(3) *In view of the results obtained in this experiment, is the electrical charge of colloidal gelatin positive or negative? Explain your answer.*

(4) *In terms of ionic action, explain what happened in each of the three test tubes when each salt was added to the gelatin.*

(5) *Account for the different rates of solation.*

Properties of Protoplasm - Emulsions and Suspensions

OBJECT *to study suspensions, emulsions, and phase reversal.*

MATERIALS

Janus green powdered dye
Microscope slide
Microscope
Stirring rod
Distilled water—10 ml.

Iodine
Olive oil
Liquid green soap
Test tubes—4
Beaker—100 ml.
Corn starch

PROCEDURE

Put 2 ml. of water in the beaker and dissolve in it 1 or 2 grains of Janus green dye. Add 1 drop of this solution to 2 ml. of olive oil in a test tube. Shake gently and observe.

(1) *Which is the continuous phase of this emulsion?*

(2) *Explain the difference between an emulsion and a suspension.*

Place a drop of olive oil in 2 ml. of water in a test tube. Shake well and observe.

(3) *What is the characteristic feature of the continuous phase?*

Add 2 drops of liquid green soap to this mixture. Shake it well and observe. Soap is an emulsifying agent.

(4) *Describe the action of an emulsifying agent.*

Place a drop of olive oil on a microscope slide. Add a drop of the Janus green dye solution. Mix with a stirring rod. Observe under low power of the microscope. Describe your observations, and explain how they illustrate phase reversal.

Place one gram of powdered corn starch in 10 ml. of distilled water, and shake thoroughly. Add 1 drop of iodine, shake, and observe. Place a drop of this mixture under the low power of a microscope and observe. Compare this to a drop of the olive oil and water emulsified with soap which you made previously.

- (5) *What is the difference between an emulsion and a suspension?*
- (6) *Explain why phase reversal is possible in an emulsion but not in a suspension.*

Water Transport in Plants

OBJECT to study xylem conduction and transpiration in higher plants.

MATERIALS

Stalk of celery with leaves or a cutting of <i>Impatiens</i>	Geranium—(<i>Pellargonium</i>)
Safranin dye, 1% aqueous solution	Wandering Jew (<i>Zebrina</i>)
Razor blade or scalpel	Distilled water
Microscope slides—2	Blotting paper
Glass tumblers—10 or	Cardboard
Beakers 250 ml.—10	Adhesive tape 1 1/2" wide
Vaseline	Beaker—100 ml.
	Microscope
	Vacuum wax

PROCEDURE

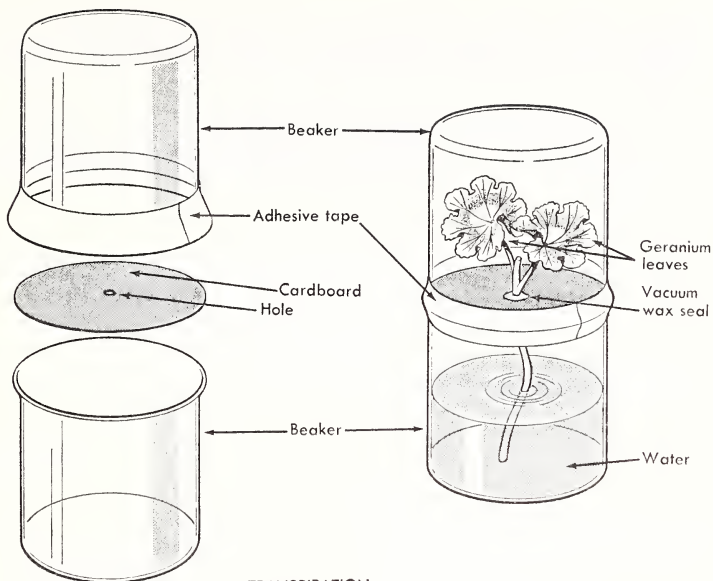
Conduction Put 50 ml. of water into a beaker, and add 15 drops of safranin dye. Cut off the lower end of the celery or *Impatiens* petiole under water, and immerse it in the beaker of dye. Place the preparation in a sunny location, and let it stand for 1/2 hour or more. While waiting proceed with the next part of this exercise. At the end of this time cut thin sections from the petiole at various distances from the original cut. Observe sections under low power and look for evidence of staining in the vascular bundles.

(1) Describe the location of the dyed tissues. What tissues are these?

Transpiration Cut a piece of cardboard to fit the top of a tumbler or beaker. Make a small hole in the centre. Put the stem of a fresh cutting of geranium bearing several leaves through the hole so that the cut end of the stem is immersed in water in the tumbler. Seal the space around the stem with vacuum wax. Cover the leaves with a second tumbler. Seal the two together with adhesive tape. Place the apparatus in a bright, cool place; fluorescent lighting is good.

As a control, set up a similar pair of tumblers in which the leaves have been removed from the stem. Observe and record your results.

Arrange three additional pairs of tumblers as outlined above, and number them. In the first one, apply vaseline to the upper surface of the leaves. In the second one, apply vaseline to the lower surfaces of the leaves. In the third, apply vaseline to both leaf surfaces. Place all three in bright light in a cool place and observe at 10 minute intervals. Record the results in table form.



TRANSPIRATION

FIGURE 1. TRANSPIRATION

- (2) *Explain the results you obtained.*
- (3) *How does illumination influence transpiration? Is there any adaptive significance in this?*
- (4) *How does humidity of the atmosphere influence transpiration? Explain your answer.*

Guard cells Detach a leaf from a geranium plant or wandering Jew (*Zebrina*) which has been well illuminated for at least one hour. Break the leaf transversely so that the two parts are hinged together by the lower epidermis. Now, pull one section of the leaf back over the other, thereby peeling off a large flap of epidermis. Immerse this stripped epidermis in a dish of water. Mount a small piece on a slide with water; cover and examine under the high power of a microscope. Are the stomates open or closed?

Put a drop of 0.4 M CaCl_2 solution on one edge of the cover slip, and draw this salt solution under the cover slip by applying blotting paper to the opposite edge. What happens to the stomates?

Replace the CaCl_2 solution under the cover slip with distilled water using the blotting-paper technique described above. Observe the stomates once more and note what happens.

- (5) *Interpret your results.*

- (6) *Guard cells contain chloroplasts, other epidermal cells do not. Can you suggest an adaptive significance of this characteristic of guard cells?*
- (7) *What are the main forces responsible for the rise of sap in trees?*
- (8) *How does sap rise in trees in early spring when leaves have not matured?*

Photosynthetic Pigments

OBJECT *to separate leaf pigments and study:*

- A. differential solubility of green and yellow pigments,*
- B. the effect of light on chlorophyll,*
- C. the effect of changing pH on anthocyanins and related pigments.*

MATERIALS

Part A

Stock solution of chlorophyll
(see appendix)
1 test tube
Benzene

Part B

Separated pigment solution from
part A
2 test tubes
10 ml. pipette

Part C

1 small beet or 5 deep-red coleus
leaves
Mortar and pestle
Fine washed sand
3 test tubes
Concentrated HCl
Filter paper
Filter funnel

PROCEDURE

Part A

Place 8 ml. of stock solution of chlorophyll (see appendix) in a 25 ml. test tube. Add an equal volume of benzene. Mix the two by inverting the tube. If the mixture does not readily separate into two parts, add water a drop at a time, shaking the tube after each addition, until the separation begins. The mixture will separate into two parts. The upper, dark green part is a solution of two kinds of chlorophyll in benzene; the lower, yellowish part is a solution of carotene and xanthophyll in alcohol and water. Save these two solutions for use in Part B.

Part B

With a pipette draw off 3 ml. of the lower, yellowish solution of carotene and xanthophyll from the tube saved from Part A. Place the liquid in a test tube, and put it in a dark place until later as a control.

In a similar manner, pipette off 5 ml. of the upper, dark green layer, and put it in the dark until later also as a control.

Place the original tube with its contents in strong sunlight or strong artificial light. Observe at 5 minute intervals, and compare with the controls previously placed in the dark.

INTERPRETATION

There is some evidence that in the living plant, chlorophyll is constantly being synthesized and decomposed. When conditions are not favorable for its synthesis, it may be destroyed faster than it is made.

- (1) *How does this experiment on chlorophyll help to explain the autumn colouration of leaves?*
- (2) *Which of the pigments studied is most sensitive to light?*

Part C

Put 15 grams of diced beets, 25 ml. of water, and 5 grams of sand in a mortar. Grind to remove beet pigment.

Filter the liquid, and divide the filtrate equally between three test tubes. Number the test tubes. To tube #1 add 2 or 3 drops of NaOH; to tube #2, add 2 to 3 drops of HCl. Leave tube #3 as a control. Record your observations in tabulated form.

Acidify the basic solution, and add some base to the acidic solution. Note that the colour change is reversible.

- (3) *What effect upon leaf colouration might one anticipate if plants containing these pigments were grown in alkaline or acidic soils? You may wish to devise an experiment to substantiate your thinking.*

Photosynthesis - Gas Exchange

OBJECT *to observe evidence of gas exchange between a green plant and its environment.*

MATERIALS

<i>Elodea</i> , sp.—8 shoots	Conc. $MnCl_2$ solution
#17 test tubes—8	Conc. NaOH solution
Brom thymol blue stock solution (see appendix)	Bunsen burner
Glass blow pipe (18" long)	Ring
#5 solid rubber stoppers—4	Ring stand
Beaker—1000 ml.	Asbestos wire gauze

PROCEDURE

Number four test tubes. Fill them about $\frac{3}{4}$ full with tap water. Add 1 ml. of brom thymol blue stock solution. Using the blow pipe, blow into the liquid until it just turns yellow. Add tap water, one drop at a time, to turn the indicator greenish. This establishes the neutral end point for this indicator.

Into tubes #1 and #2 place a 4-6 inch shoot of *Elodea* and tightly stopper all four test tubes.

Place tubes #1 and #3 in the dark and the other two test tubes in a strong light. Observe at intervals throughout the lesson period, and record your observations. If no change is observed during this time, keep the tubes under the conditions described until the next laboratory period.

Brom thymol blue is an indicator which is yellow in acid, blue in base, and greenish in the intermediate range.

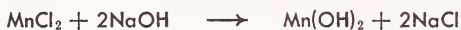
- (1) Account for any colour change in tube #1.
- (2) Account for any colour change in tube #2.
- (3) What is the purpose of using tubes 3 and 4? Explain fully.

Number four test tubes. Fill them about $\frac{3}{4}$ full of boiled and cooled water. Into tubes #1 and #2 place 4-6 inch shoots of *Elodea*. Tightly stopper all tubes. Place tubes #1 and #3 in the dark and the other two tubes in strong light.

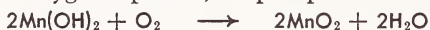
Before the end of the lesson period, remove the shoots of *Elodea* from tubes #1 and #2. Test the contents of all tubes for the presence of oxygen by adding 1 ml. of MnCl_2 solution and 1 ml. of NaOH solution to each tube. Stopper all tubes immediately.

INTERPRETATION

If concentrated manganous chloride and sodium hydroxide are added to water devoid of oxygen, a white precipitate of manganous hydroxide is formed.



If oxygen is present, the precipitate is brown.



Record your observations in table form.

- (4) *Account for the colour of the precipitate in tube #1.*
- (5) *Account for the colour of the precipitate in tube #2.*
- (6) *What is the purpose of using tubes #3 and #4? Explain fully.*

Photosynthesis - The Role of Carbon Dioxide

OBJECT *to demonstrate the role of CO_2 in photosynthesis.*

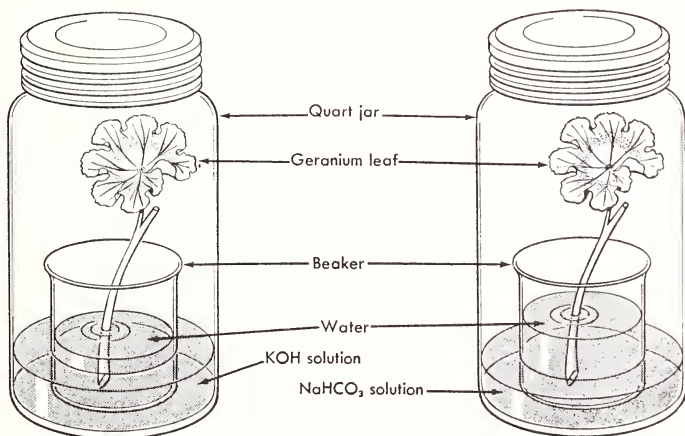
MATERIALS

A geranium plant with several leaves	5% KOH solution
2 quart jars with tight-fitting lids	Stock I, KI solution
5% NaHCO_3 solution (freshly prepared)	Petri dish

PROCEDURE

Put the geranium plant in complete darkness until all stored starch has disappeared from the leaves, i.e. for about 72 hours or until a piece of leaf gives no positive starch test.

Set up apparatus, as shown in the sketch below, with water in the beaker, KOH solution in the bottom of one jar, and NaHCO_3 solution in the bot-



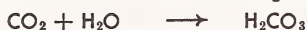
PHOTOSYNTHESIS—CARBON DIOXIDE

tom of the other. Do not allow either solution to touch the leaves or to get into the beakers of water.

Tightly close both jars and keep them exposed to strong light until next day. Then test both leaves for starch.

INTERPRETATION

KOH solution absorbs CO_2 from the air in the jar.



Sodium bicarbonate decomposes to provide an enriched CO_2 atmosphere.



From your observations of this demonstration, interpret the results.

Digestion - Carbohydrates

OBJECT *to study the effects of enzymes and pH on carbohydrates.*

MATERIALS

Part A

#6 test tubes—10
 Test tube racks—2
 1% starch suspension—8 ml.
 Pancreatin solution—4 ml.
 (see appendix)
 Saliva—4 ml.
 Beaker—100 ml.
 0.2% NaOH solution—3 ml.
 I, KI solution
 Bunsen burner
 Incubator
 Benedict's solution

Part B

#6 test tubes—4
 1% starch suspension—4 ml.
 1% sucrose solution—4 ml.
 10% HCl solution—2 ml.
 Benedict's solution
 Beaker—400 ml.
 Ring stand
 Asbestos wire gauze
 Bunsen burner

PROCEDURE

Part A

Enzymic digestion Number ten test tubes. Add 2 ml. of pancreatin to tubes #1 and #2 and 2 ml. of saliva to tubes #4 and #5. Boil tubes #2 and #5 over a bunsen burner for one minute. Cool, and then add 2 ml. of starch suspension to tubes 1-5 inclusive. To tubes #1, #2, and #3 add 1 ml. of 0.2% NaOH solution. Shake well, and incubate these tubes at 37°C. for 15 minutes.

(1) *Compare the appearance of the contents of these tubes before and after incubation, and explain what happened in each tube.*

Shake each tube well, and pour half the contents of tubes #1 to #5 into tubes #6 to #10 respectively. Test the contents of tubes #1 to #5 for starch (iodine test) and tubes #6 to #10 for reducing sugars (Benedict's test). Record the results of these tests in a table similar to the one on the following page.

Tube number	Results
1 pancreatin, NaOH, starch	
2 pancreatin (boiled), NaOH, starch	
3 starch, NaOH	
4 saliva, starch	
5 saliva (boiled), starch	
6 pancreatin, NaOH, starch	
7 pancreatin (boiled), NaOH, starch	
8 starch, NaOH	
9 saliva, starch	
10 saliva (boiled), starch	

(2) Account for the results obtained in the iodine and Benedict's tests as recorded in the above table.

(3) Why was NaOH added to tubes #1, #2, and #3?

Part B

Hydrolysis Prepare a water bath using a 400 ml. beaker. (See appendix B.) Number four test tubes. To tubes #1 and #2 add 2 ml. of 1% starch suspension. To tubes #3 and #4 add 2 ml. of 1% sucrose solution. Add 5 drops of 10% HCl to tubes #1 and #3. Place all four tubes in the water bath, and boil for ten minutes. Cool and test the contents of each tube for reducing sugars (Benedict's test). Record your results in a table: Column headings: *Tube number, Results*. Line headings: *Starch – HCl, Starch, Sucrose – HCl, Sucrose*.

Digestion - Proteins

OBJECT *to study the effects of enzymes and pH on proteins.*

MATERIALS

Part A

#6 test tubes—3
 Test tube rack
 Rennin junket stock solution (fresh, see appendix)
 Powdered skim milk stock solution (see appendix)
 Bunsen burner

Part B

#6 test tubes—5	Brom thymol blue dye
Test tube rack	Beaker—100 ml.
Gelatin powder—1 gram	Stirring rod
Distilled H ₂ O	Medicine dropper
10% HCl	Bunsen burner
10% NaOH	Test tube holder
Pepsin stock solution—10 ml.	Tongs
	Incubator

PROCEDURE

Part A

Number three test tubes. Add 1 ml. of rennin stock solution to tubes #1 and #2. Boil the contents of tube #2 over a bunsen burner for one minute and cool. Add 2 ml. of powdered skim milk stock solution to all three test tubes, and incubate at 37°C. for ten minutes. Observe and record the results in tabulated form. Interpret the results in all three test tubes.

Part B

Number five test tubes. Add 2 ml. of pepsin stock solution to test tubes #1 to #4 inclusive. Boil the pepsin in tube #4 over a bunsen burner for 1 minute.

Dissolve 1 gram of powdered gelatin in 20 ml. of distilled water. Heat gently to dissolve the gelatin, but do not boil. Cool to luke warm, and add

2 ml. of the dissolved gelatin and 5 drops of brom thymol blue dye to each of the five tubes. Add 1 drop of 10% HCl to tubes #1 and #5. To tube #2 add 2 drops of 10% NaOH. (Blue colour appears when the solution is alkaline).

Incubate at 37°C. for 20 minutes. Remove from the incubator, and cool all tubes in ice water. Record your observations in a table similar to the one below.

Tube number	Observations
#1 gelatin, HCl, pepsin	
#2 gelatin, NaOH, pepsin	
#3 gelatin, pepsin	
#4 gelatin, pepsin (boiled)	
#5 gelatin, HCl	

Interpret the results in all tubes. Refer back to your notes on Lab. 8.

Digestion - Fats

OBJECT *to study the effects of enzymes and pH on fat digestion and illustrate complete digestion of milk.*

MATERIALS

Part A

#6 test tubes—9
 Test tube racks—2
 10% HCl
 10% NaOH
 Brom thymol blue
 Pancreatin stock solution
 Bunsen burner
 Incubator (see appendix)
 I, KI solution (see appendix)
 CCl₄—8 ml.
 KHSO₄—8 grams
 Emulsifying agent (bile salts, detergent,
 or green soap) (if time permits try
 each in turn)
 Olive oil—8 ml.

Part B

#6 test tube
 10% HCl
 10% NaOH
 Brom thymol blue
 Pancreatin stock solution
 Powdered skim milk solution—4 ml.

PROCEDURE

Part A

Number four test tubes, and add 2 ml. of olive oil and 5 drops of brom thymol blue dye to each tube. To tube #1 add 5 drops of emulsifying agent, 2 ml. of pancreatin stock solution, and 10% NaOH dropwise until a blue-green colour appears. To tube #2 add 5 drops of 10% HCl and 2 ml. of pancreatin. To tube #3 add 5 drops of HCl, and to tube # 4 add 5 drops of 10% NaOH. Incubate all tubes at 37°C. for 15 minutes. While waiting proceed with part B.

After incubation observe contents of all tubes, and explain any changes. Split the contents of tubes 1-4 into tubes numbered 1A, 2A, 3A, and 4A respectively.

Test the contents of tubes 1-4 for fatty acids by adding 2 ml. of CCl₄ and 10 drops of I, KI solution to each tube. Shake well. Now, add 10 drops

of saturated HgCl_2 solution to each tube. Shake well, and compare the colours of the contents of these tubes with a control tube containing 2 ml. of olive oil, 2 ml. of CCl_4 , 10 drops of I, KI, and 10 drops of HgCl_2 solution. A yellow flocculum indicates the presence of fatty acids; salmon red colour otherwise. Record results in a table similar to the one below.

To tubes 1A-4A add 2 grams of solid KHSO_4 . Heat over a bunsen burner. Smell cautiously. The pungent odour characteristic of acrolein indicates the presence of glycerin.

Tube number		Observations and results
1	olive oil, NaOH, detergent, pancreatin	
2	olive oil, HCl, pancreatin	
3	olive oil, HCl	
4	olive oil, NaOH	
1A	olive oil, NaOH, detergent, pancreatin	
2A	olive oil, HCl, pancreatin	
3A	olive oil, HCl	
4A	olive oil, NaOH	

- (1) *Gastric lipase is present in the stomach; however, little or no fat digestion takes place there. Explain.*
- (2) *Explain from the results of this experiment why, in man, most fat digestion takes place in the small intestine.*

Part B

To a test tube add the following: 4 ml. of milk, 2 ml. of pancreatin, 5 drops of brom thymol blue, and 2 drops of HCl. Incubate it for 20 minutes. After incubation, observe and record any changes. Shake well, and add, drop by drop, enough NaOH to just alkalize. Incubate until the end of the period, shaking the contents of the tube at intervals. Record your results under the following headings: *Results of incubating acid medium, Results of incubating alkaline medium.*

- (3) *How does this experiment illustrate complete digestion?*

Respiration - Gas Exchange

OBJECT *to study gas exchange and breathing control.*

MATERIALS

Part A

100 ml. graduate cylinders—2
Neutral red dye
Glass tubing 18"
Aquarium air-breaker stone—1

$\frac{1}{8}$ " rubber tubing—1"
 $\text{Ba}(\text{OH})_2$ solution
HCl (concentrated)
Lime water—50 ml.
10% NaOH solution

Part B

#8 paper bags—1 per student

PROCEDURE

Part A

Add 50 ml. of lime water to a 100 ml. graduate cylinder. Arrange a blow pipe as shown in the sketch.

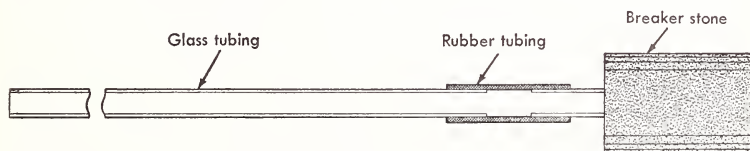


FIGURE 3. RESPIRATION—GAS EXCHANGE

Using the blow pipe, bubble exhaled air through the lime water. The appearance of a milky precipitate indicates the presence of CO_2 in the exhaled air.

- (1) Write balanced chemical equations to show these reactions. As a confirmation test, add a few drops of concentrated HCl. The solution should become clear.
- (2) Write a balanced chemical equation for the reaction.

Add 50 ml. of tap water to a 100 ml. graduate cylinder, and add 10 drops of neutral red dye. This dye is a yellow-orange colour in a basic solution and red in acids. If necessary, adjust the pH of the solution to where it is just basic by adding a drop of 10% NaOH.

Using the blow pipe from Part A, bubble exhaled air through the contents of the graduate cylinder.

(3) *Account for the colour change.*

(4) *In view of the results of this experiment and the fact that CO_2 is constantly produced in the cells of the body, why does protoplasm not become highly acidic?*

Part B

Work in pairs.

These exercises demonstrate the effects of CO_2 concentration on the rate of breathing. One partner should serve as a test subject while the other records data and keeps the time. Alternate these roles in each test. Record results of each test for both partners; count one breath as the time required for one inhalation and one exhalation.

Count the number of breaths per minute during normal breathing while sitting at rest. Record the average of three one-minute trials.

Take very deep, forced breaths for one minute at a faster than normal rate. After this "forced ventilation", breathe as normally as possible. Count and record the number of breaths per minute.

Hold a paper bag tightly over your nose and mouth so that you are forced to rebreathe the same air. Do this for two minutes. Breathe as normally as possible. Count and record the number of breaths during the second minute.

Stand and quickly do 25 knee bends. Sit quietly while your partner counts and records the number of unforced breaths per minute.

After resting and breathing normally for one minute take a deep breath and hold it as long as possible. Record the time the breath was held.

After hyperventilating as in step 2 above, again determine the length of time a deep breath can be held.

Make a record in table form showing results for each partner. Column headings will show the activities as follows: Rate of normal breathing while at rest; Rate of breathing after forced ventilation; Rate of breathing after rebreathing same air; Rate of breathing after exercise; Time breath can be held after resting; Time breath can be held after hyperventilating.

(5) *Describe the regulating mechanisms in the body which ensure that breathing continues automatically and without conscious effort.*

(6) *Account for your data on breathing rate after forced ventilation.*

(7) *Account for your data on breathing rate after exercise.*

(8) *Account for your data on breathing rate after rebreathing the same air.*

- (9) *Account for your data on variation in breath-holding ability under different conditions.*
- (10) *Why is it impossible to hold one's breath for very long under any conditions?*

Respiration - Aerobic

OBJECT *to study aerobic respiration in germinating seeds.*

MATERIALS

Viable seeds—50 ml.
(wheat, oats, radish, etc.)
Florence flask—300 ml.
One-hole stopper to fit flask
Delivery tube
250 ml. beaker
Colouring

Ring stand
Burette clamp
Concentrated NaOH—10 ml.
Test tube (13 x 100 mm.—no lip)
Vacuum wax

PROCEDURE

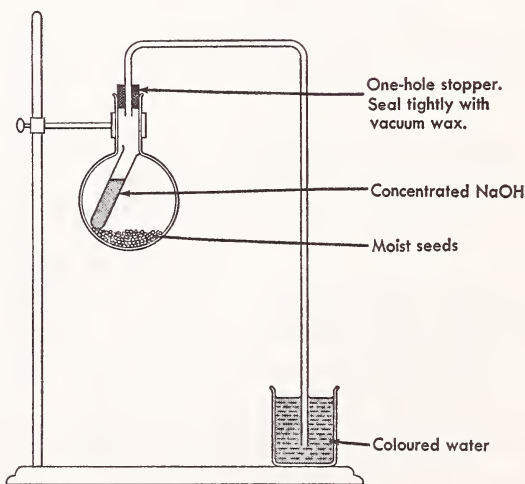


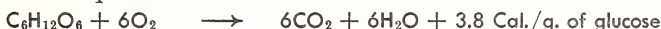
FIGURE 4. RESPIRATION—AEROBIC

Put 50 ml. of dry viable seeds into a 300 ml. florence flask. Add enough water to moisten all seeds. Add 10 ml. of concentrated NaOH to a 13 x 100

mm. test tube, and carefully lower it into the Florence flask as shown in the diagram. (Do not spill the NaOH on the seeds.) Assemble the apparatus as shown. Observe and mark the level of the coloured water in the delivery tube (a) at the beginning of the experiment, (b) at the end of the period, and (c) 24 hours later.

INTERPRETATION

Aerobic respiration in germinating seeds requires oxygen for oxidative metabolism. This reaction may be represented by the following simplified chemical equation:



The CO_2 evolved by the germinating seeds is removed by the NaOH solution in the small test tube. Since the seeds evolved as much CO_2 as they used O_2 , the decrease in pressure in the flask, indicated by a rise of coloured water in the delivery tube, is an indirect measure of the O_2 consumed.

- (1) *How could one devise a suitable control for this experiment? Fully explain the theory involved in this control experiment.*
- (2) *Discuss the statement, "Aerobic respiration in plants and animals is essentially the same."*

Respiration - Aerobic and Anaerobic

OBJECT *to study respiration in baker's yeast.*

MATERIALS

Yeast-glucose suspension—60 ml.

(see appendix)

#6 test tubes—4

Test tube rack

Test tubes (13 x 100 mm.) no lip—2

(to fit snugly inside a #6 test tube)

Hand centrifuge desirable

Incubator desirable

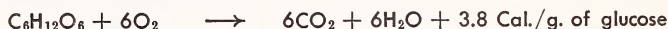
1 M NaF solution—3 ml.

10% NaOH solution—5 ml.

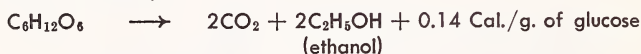
Lugol's solution—5 ml.

(see appendix)

Yeast is a valuable organism in which to study respiration because it respire aerobically:



and anaerobically:



If oxygen is available, yeast cells will respire aerobically and anaerobically at the same time. As the supply of available oxygen changes, one of the processes will be accentuated.

PROCEDURE

Carbon dioxide production To prepare a respirometer, fill a 13 x 100 mm. test tube with yeast-glucose suspension. Hold a #6 test tube upside-down and slide it over the yeast-filled test tube until the rounded bottom of the large test tube comes to rest against the open end of the small tube. Holding the tubes in this position, quickly invert the entire system. The trapped air space in the small tube should be kept as small as possible. Use a wax marking pencil to mark the size of the air space.

Prepare a second respirometer in the same way, but add 1 ml. of 1 molar sodium fluoride to the test tube before filling with yeast-glucose suspension. Assemble as before.

CAUTION: *Fluorides are poisons and must be handled carefully.*

Place both respirometers in an incubator at 37°C. for 24 hours, and then test for ethyl alcohol according to the following procedure: (iodoform test). Place 5 ml. of the ferment from each tube in two separate test tubes. Number the tubes for identification.

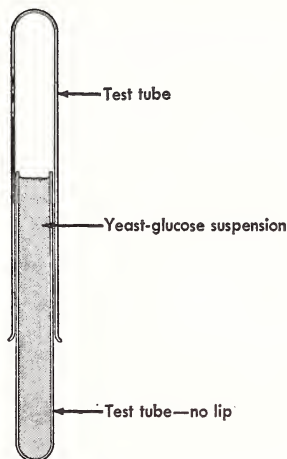


FIGURE 5. RESPIRATION—AEROBIC AND ANAEROBIC
CARBON DIOXIDE PRODUCTION

To each tube add 4 drops of 10% NaOH and Lugol's solution dropwise until the solution just changes colour. Centrifuge or allow to stand for 10 minutes. The formation of a yellow precipitate (iodoform) on the bottom of the test tube indicates the presence of ethyl alcohol. Record the results.

INTERPRETATION

In the respiratory oxidation of glucose by yeast, one of the intermediate steps is the conversion of phosphoglyceraldehyde (PGAL) to pyruvic acid. (See Weisz, page 261.) Pyruvic acid is subsequently transformed aerobically into CO_2 and H_2O or anaerobically into CO_2 and ethyl alcohol ($\text{C}_2\text{H}_5\text{OH}$).

The conversion of PGAL to pyruvic acid (more than one step is involved) requires specific enzymes. One of these enzymes is enolase. This enzyme functions only if magnesium ions are available. Sodium fluoride will precipitate the magnesium ions present in dry baker's yeast thus blocking respiration at this point.

- (1) *What was the precipitate which was formed in the test tubes to which NaF was added?*
- (2) *Account for any significant difference in gas production in the two respirometers.*

- (3) *Explain why fluorides and cyanides are poisonous.*
- (4) *Considering the experimental conditions in the respirometers, did the yeast cells respire aerobically or anaerobically? Explain.*

Respiration - Dehydrogenation

OBJECT *to study hydrogen transfer and dehydrogenation.*

MATERIALS

Methylene blue dye 0.05% solution	Test tube rack
Yeast-glucose suspension (see appendix)—45 ml.	Incubator
#6 test tubes—3	NaF—one molar solution—1 ml.
	Formaldehyde (10% solution)—1 ml.

PROCEDURE

Number three test tubes. To each tube add 15 ml. of yeast-glucose suspension and 0.5% methylene blue solution dropwise until the mixture is light blue. To tube #1, add 1 ml. of 1 molar NaF solution. To tube #2 add 1 ml. of 10% formaldehyde solution. Incubate all tubes at 37°C., and observe at five-minute intervals. Record results after a significant effect is established in tube #3. After recording results, thoroughly shake contents of tube #3. Observe and record results.

INTERPRETATION

Methylene blue has the property of being able to combine with hydrogen. It becomes colourless in the process and can be *re-oxidized*, at which time it will regain its blue colour. It is therefore possible to use methylene blue as a hydrogen acceptor and to gauge the chemical change of the dye by the colour change. If no oxygen is available, the methylene blue can be used as an indicator of dehydrogenation of fuels and hence of respiration.

- (1) *Account for all colour changes in tube #3. Why did the colour change first in the lower regions of this tube when it was incubated?*
- (2) *Account for the results in tubes one and two.*

Respiration - Anaerobic

OBJECT *to study anaerobic respiration in yeast.*

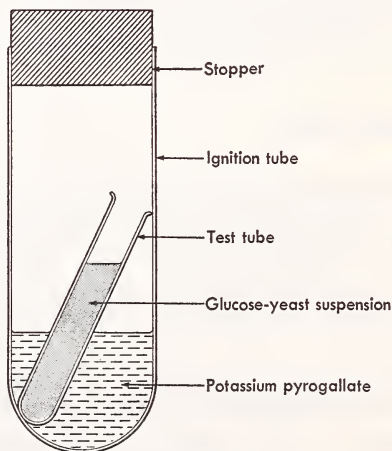
MATERIALS

#6 test tubes—2
Culture tube—13 x 100 mm.
Ignition tubes—25 x 200 mm.—2
#5 solid rubber stoppers—2
Potassium pyrogallate solution
—50 ml. (see appendix)

Yeast-glucose suspension—40 ml.
0.05% methylene blue solution
Lugol's solution—5 ml.
Incubator
Hand centrifuge desirable
(see appendix)

PROCEDURE

FIGURE 6.
RESPIRATION—ANAEROBIC



Prepare one respirometer as outlined in Lab. #19.

To the glucose-yeast suspension in the respirometer add 0.05% methylene blue solution dropwise until the mixture is light blue.

Put 15 ml. of glucose-yeast suspension in a #6 test tube.

Put 25 ml. of potassium pyrogallate (see appendix) into each of the two ignition tubes.

Place the respirometer in one ignition tube and the test tube of glucose-yeast suspension in the other. Tightly stopper both ignition tubes (see fig. 6). Incubate both tubes at 37°C. Observe at 10 minute intervals during the period and after 24 hours. Record observations at the end of the period and after 24 hours. After 24 hours of incubation, test the contents of the test tube containing the glucose-yeast suspension for the presence of ethyl alcohol. (iodoform test — see Lab. 19).

- (1) *What gas was produced in the respirometer?*
- (2) *In aerobic respiration of yeast, how many molecules of oxygen are consumed for every molecule of CO_2 given off?*
- (3) *Drawing on information gained from this and preceding exercises, devise an experiment which would demonstrate that yeast respiration liberates energy in the form of heat. (Work in groups of four.)*

Body Fluids - Blood

OBJECT *to study blood cells and the function of hemoglobin.*

MATERIALS

Prepared slides of human blood	Ignition tube
Microscope	Rubber tubing $\frac{1}{8}$ " I.D.—2'
Microscope slides	Pinch clamps—2
Cover slips	Glass tubing $\frac{1}{8}$ " O.D.—2'
KClO ₃ —5 grams	Bunsen burner
MnO ₂ —1 gram	Whole blood, defibrinated (see appendix)
CaCO ₃ —5 grams	Air breaker stones—2
Concentrated H ₂ SO ₄ —10 ml.	Erlenmeyer flasks, 250 ml.—2
Florence flask, 300 ml.	Acetic acid 4%
Rubber stopper, two-holed	
Human Ringer's solution	

PROCEDURE

Blood cells Place a drop of whole blood on a clean slide, and add a drop of Ringer's solution (see appendix) to it. Cover the wet mount with a cover slip, and examine it under the microscope. Locate both white and red blood cells, and note the vast number of red cells in comparison to white blood cells.

Destroy the red blood cells by placing a drop of 4% acetic acid at the edge of the cover slip so that it diffuses into the mounting fluid. A clear field of white blood cells should remain behind.

(1) *What is the approximate ratio of the number of red blood cells to white blood cells?*

Examine prepared mounts of blood under the microscope. Look for both red blood cells and white blood cells.

(2) *Are all the white blood cells the same? If not, explain.*

(3) *Do the red blood cells contain nuclei?*

(4) *Describe the functions of red blood cells and white blood cells.*

(5) *How can you prevent blood from clotting if it is to be stored for transfusions?*

Gas transport in blood Prepare two Erlenmeyer flasks, each with a short piece of glass tubing to which an air breaker stone is attached and a right-angle bend of glass tubing in a rubber stopper. Rubber tubing leads from the bend to an oxygen generator (see appendix) from one flask, and a carbon dioxide generator (see appendix) from the other.

Record the colour differences which develop between the two flasks as the gases are bubbled through the blood. (See fig. 7.)

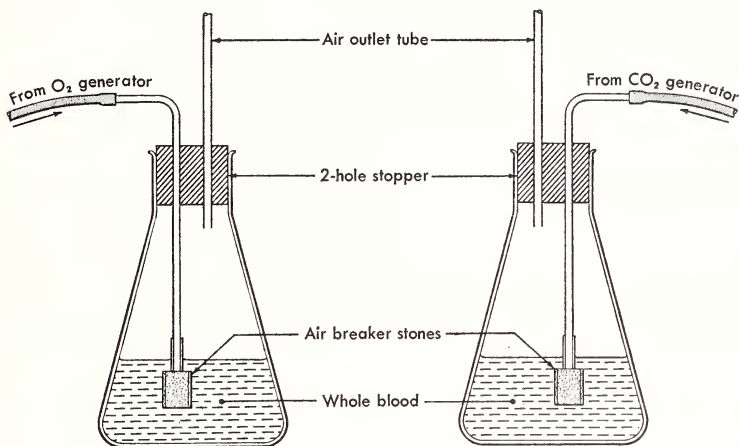


FIGURE 7. BODY FLUIDS—BLOOD

(6) *What chemical event is taking place in each flask?*

Clamp the delivery tubes and reverse the attachments so that the oxygen now flows into the flask which formerly received the CO_2 and the CO_2 now flows into the flask which previously received the oxygen. Record the colour change in the blood in each flask.

(7) *How do the colour differences here help to explain the differences between arterial or aerated and venous or unaerated blood?*

The binding and freeing of oxygen by hemoglobin can be represented by the following equilibrium:



(8) *Explain how and why this equilibrium is shifted (a) while the blood flows through the capillaries of the lungs and (b) while the blood flows through the capillaries of other parts of the body.*

Carbon monoxide is an extremely poisonous gas; 1 part in 1500 parts of air will prove fatal. It combines with Hb to form carboxyhemoglobin which cannot take up oxygen. In treating victims of CO poisoning, arti-

ficial respiration is first applied in the open air, followed by an inhalation of a mixture of 93% oxygen and 7% carbon dioxide.

(9) *What is the purpose of using this concentration of O_2 and of giving CO_2 at the same time? Explain your answer fully.*

Blood Types

OBJECT *to determine blood types.*

MATERIALS

Microscope
Microscope slides—4
Wax pencil
Sterile disposable blood
lancets—4

Beaker, 250 ml. containing 75%
ethanol and water
Absorbent cotton
Blood typing sera, anti-A and anti-B
Vial containing 4 sterilized toothpicks

PROCEDURE

(Work in groups of four. Each student is to carry out the complete exercise.)

Mark the slide as shown in fig. 8a. At the left, place a drop of anti-A serum; at the right, a drop of anti-B serum.

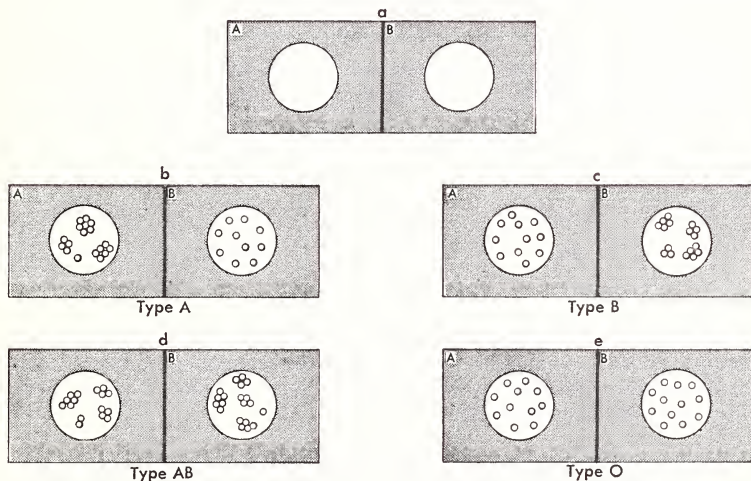


FIGURE 8. BLOOD TYPES

Sterilize the tip of the second finger. Use absorbent cotton dipped in the alcohol. Allow at least one minute for sterilization.

CAUTION: *Do not use anything other than the lancet provided for puncturing. Wipe the skin with 75% ethanol before puncturing. Never use a lancet more than once. Those provided are sterile – do not open the package before you are ready to use them, and do not allow the cutting point to come in contact with anything before use.*

Puncture the finger. Do not be gentle or you will not get any blood. Squeeze a drop of blood to the surface of the puncture.

With one end of the toothpick provided, transfer a drop of blood to the drop of anti-A serum on the slide and stir it. With the *other end* of the toothpick transfer a second drop of blood into the drop of anti-B serum. Tilt the slide back and forth gently and note the immediate reaction (unless the blood is type O). Examine under low power of the microscope.

Record the results, and check them against those in the illustrations.

INTERPRETATION

If blood of unknown type is mixed with anti-A serum and clumping of the corpuscles occurs, the unknown blood must be type A (fig. 8b). Similarly, if the unknown blood clumps in anti-B serum, the unknown blood belongs to group B (fig. 8c). Should the unknown blood clump in both drops of serum, anti-A and anti-B, the unknown blood belongs to type AB (fig. 8d). O type blood, which lacks the two antigens in the red blood cells, will not clump in either serum (fig. 8e).

Blood groups	Agglutinogens in corpuscles	Agglutinogens in plasma	Can donate to	Can receive from
A	A	b	A,AB	O,A
B	B	a	B,AB	O,B
AB	A and B	none	AB	O,A,B,AB
O	none	a and b	O,A,B,AB	O

While the numbers of people with each blood type vary among racial groups within a population, very roughly the frequency of blood types in the western hemisphere is as follows: Type O – 45%, Type A – 42%, Type B – 10%, Type AB – 3%.

- (1) *Explain why it is that a person of blood type “O” can donate blood to persons of other blood types but can receive whole blood from an “O” donor only.*

- (2) *In addition to the agglutinogens listed in this exercise, what other agglutinin must be considered when transfusions of whole blood are proposed? Explain.*
- (3) *Determine the number of students in your class who have each type of blood. Do these figures approximate the percentage distribution given above? Suggest factors which may account for any differences.*

Blood Circulation - Capillary Flow and Heart Rate

OBJECT *to examine blood flow in a capillary and to study the effects of various stimuli on the rate of the heart beat.*

MATERIALS

Microscope	Dissecting scissors—1 pair
Cheese cloth—1 piece 6" x 12"	Dissecting probe
Thread	Forceps—1 pair
Pins	Test tube holders—2
Adrenalin, (1:10,000)—5 ml.	Medicine droppers—2
Acetylcholine, (1:10,000)—5 ml.	Watch with second hand
Live pithed frog (see appendix)	
Frog pinning board (see appendix and fig. 9)	
Frog Ringer's solution (see appendix)—10 ml. iced; 30 ml. at room temperature; 10 ml. at 40°C.	

PROCEDURE

Capillary flow Wrap a pithed frog in wet cheese cloth. Leave one hind leg exposed, and attach to a frog pinning board as shown in figure 9.

Clamp the prepared pinning board to the stage of a microscope with two test tube holders. Examine the capillaries in the web of the foot under low power.

- (1) *How large are the blood cells in relation to the diameter of the capillaries?*
- (2) *Describe the manner of blood flow observed.*
- (3) *What is the function of the capillary blood?*

Heart beat Remove the pithed frog from the pinning board, and remove the cheese cloth. Fasten the frog, ventral side up, by pinning through the extended limbs. Using dissecting scissors, slit the skin, muscles, and pectoral girdle along the mid line, from the epigastrium to the chin, and thus expose the heart. Pick up the pericardium, and slit it carefully with the scissors. It is important that all exposed tissues be kept wet with frog

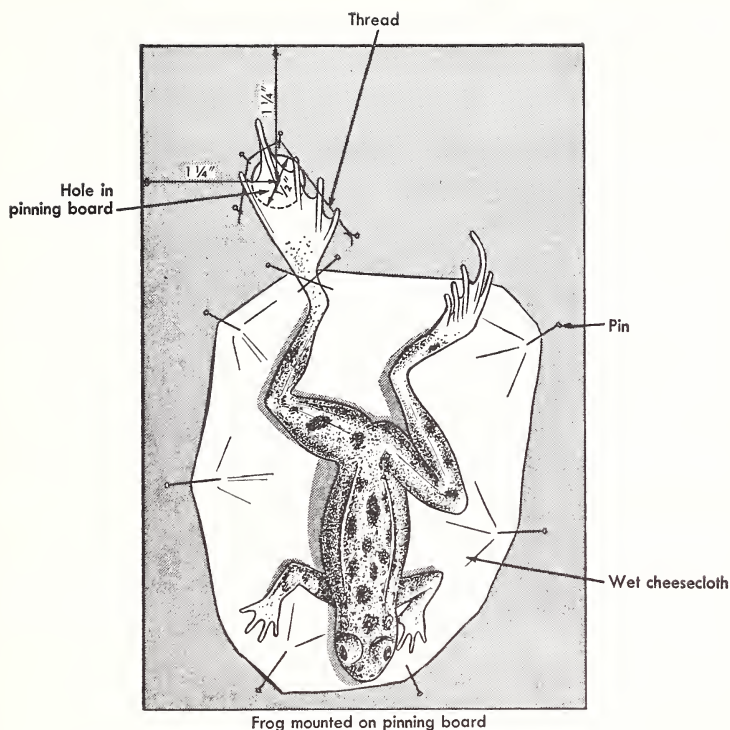


FIGURE 9. BLOOD CIRCULATION—CAPILLARY FLOW AND HEART RATE

Ringer's solution. Use a medicine dropper to apply the solution. Record the number of heart beats per minute.

Flood the heart and surrounding tissues with iced Ringer's solution for one minute, and then again count and record the number of heart beats per minute.

Repeat the flooding procedure using the Ringer's solution at 40°C. Allow time for the heart tissues to warm. Count and record the number of heart beats per minute.

Flood the heart with Ringer's solution at room temperature to restore normal rate of heart beat. Repeat the flooding procedure using (a) adrenalin solution and (b) acetylcholine solution. Record the number of heart beats per minute with each.

- (4) *In view of the results of this experiment, explain how heart-beat is affected by: (a) fever, (b) fear or anger, (c) adrenalin, (d) acetylcholine.*
- (5) *Why did the heart continue to beat after the brain and spinal cord were destroyed?*

Blood Circulation - Pulse Rate and Blood Pressure

OBJECT to study the effect of exercise on pulse rate and blood pressure.

MATERIALS











Stethoscope
Sphygmomanometer

Watch with second hand

PROCEDURE

Pulse rate Place the forefinger over the radial artery (in wrist). Count and record the pulse rate while sitting at rest and again after 10 rapid deep knee bends.

FIGURE 10. BLOOD PRESSURE

Shape of blood vessel during Systole	Shape of blood vessel during Diastole	Typical cuff pressure (mm. Hg.)	Sound heard	Designation of pressure level
		130	None	
<hr/>				
		120	"Tap" First detected	→ Systolic
<hr/>				
		100	"Swish"	
<hr/>				
		80	"Swish" longer, may become muffled	
<hr/>				
		70	None	→ Diastolic

- (1) *Is there any significant difference in pulse rate between the sexes while sitting at rest and after exercise?*
- (2) *What initiates the increased rate of heart beat during exercise? Explain fully.*

Blood pressure Blood pressure may be measured by using a sphygmomanometer and a stethoscope. The principle of this measurement is elaborated in Fig. 10.

Using the following procedure, measure and record systolic and diastolic blood pressures while at rest and again after 10 deep knee bends.

Place the arm band or cuff of the sphygmomanometer around the upper arm, making sure that clothing does not impede circulation and that the cuff is well above the elbow. Place the bell of the stethoscope over the brachial artery at the upper end of the inner surface of the forearm (Antecubital space). No sound should be heard through the stethoscope unless the bell is being pressed too firmly.

Raise the cuff pressure to 150 mm. of mercury. Slowly release the pressure and record the pressure level at which sounds are first heard (systolic blood pressure) and at which the sounds disappear (diastolic blood pressure).

Record both figures for each member of the group. The "blood pressure" is expressed as the systolic pressure over the diastolic pressure — for example 120/70.

- (1) *Account for systolic and diastolic blood pressures.*
- (2) *What blood pressures are considered normal for a person twenty-one years of age?*
- (3) *What factors may cause an increase in blood pressure?*
- (4) *What can a physician tell from blood pressure data?*
- (5) *Is the amount of the difference between systolic and diastolic blood pressure in any way informative? Explain.*

Nervous Coordination - Reflex Arcs

OBJECT *to study reflex action.*

MATERIALS

Tank of water, 10 gals. approx.—	0.5% H_2SO_4 —10 ml.
1 per class	Stirring rod
Live frog, normal	Forceps—1 pair
Spinal frog (see appendix)	Frog pinning board

PROCEDURE

Blinking reflex Hold a normal live frog securely in one hand by the hind legs. Gently touch one eye with a clean stirring rod. Carry out the same procedure with a spinal frog. Note and record the results.

Croaking reflex With a stirring rod, gently scratch the skin of a normal live frog along the anterior half of the spinal column. Repeat this procedure on a spinal frog. Record the results.

- (1) *Account for the results of the croaking reflex experiment.*
- (2) *What normal function of the cerebrum of the brain can be inferred from the croaking reflex experiment?*

Scratching reflex Grasp the head and forelimbs of a normal live frog securely in one hand, and wash off any mucus present. Now touch the dorsal skin with a stirring rod dipped in 0.5% H_2SO_4 . Observe the frog's reactions. Wash off the acid, and then record your results. Repeat the procedure using a spinal frog.

Swimming reflex Place both the normal and spinal frogs in the tank of water provided for this purpose. Observe for several minutes. Note the posture and swimming responses. Record your observations.

Jumping reflex (Use the spinal frog only) Put the spinal frog in a sitting position on the floor. (a) prod the animal from behind; (b) pinch its toe or thigh lightly with forceps.

- (3) *Did the degree of stimulation required to produce the jumping response vary?*

Balancing reflex Put a live normal frog on a frog pinning board, and tilt the board gradually. Study the balancing movements of the frog. Manoeuvre the board in such a way as to make the animal climb upwards, perch on the edge, and, as you continue to turn the board, climb down head first on the opposite side. Repeat this procedure using the spinal frog. Record your results.

(4) *With which of the frogs was this manoeuver possible? Interpret your results.*

Using the technique demonstrated by your instructor, pith the spinal frog. Repeat all the previous experiments using the pithed frog, and record the results.

(5) *Describe the general composition of a reflex arc.*

(6) *What is a nerve impulse, and how is it transmitted across a synapse?*

(7) *On the basis of the results of these experiments, what conclusions can be drawn as to the function of the spinal cord in reflex behavior?*

Nervous Coordination

OBJECT *to study the effects of cellular excitation.*

MATERIALS

Spinal frog	1% CaCl_2 solution—25 ml.
Dissecting scissors—1 pair	Beakers, 50 ml.—4
Dissecting tray	Thread—6"
Blunt probe	Hook (bent pin)
Dissecting pins	1.5 volt dry cell
Frog Ringer's solution—200 ml.	Copper wire, 18–22 gauge insulated
Medicine dropper	—2 pcs. each 1 1/2' long
Absorbent cotton	Watch with second hand
0.7% NaCl solution—25 ml.	Adrenalin—1:10,000 parts
0.9% KCl solution—25 ml.	Hypodermic syringe, 2 ml.

PROCEDURE

Heart stimulation Expose the frog's heart as outlined in Lab. 24. Flood the heart and surrounding tissues with frog Ringer's solution. Quickly but carefully remove the heart, and suspend it, by means of a piece of thread and a pin bent like a fish hook, in a 50 ml. beaker containing 25 ml. of frog Ringer's solution. Count and record the number of heart beats per minute.

Transfer the heart into a 50 ml. beaker containing 25 ml. of 0.7% NaCl solution. Count and record the number of heart beats.

Replace the heart in frog Ringer's solution for two minutes, and then transfer it to a beaker of 0.9% KCl solution. Record the time it takes for the heart to stop contracting.

Now put the heart into a 1% calcium chloride solution. The heart should begin contracting again. Replace the heart in frog Ringer's solution, and watch the return to normal rate of heart beat.

Apply a very mild electrical stimulus to the heart, by means of an insulated copper wire attached to a dry cell. Observe and record the results. Replace the heart in the beaker of 0.9% KCl, and again arrest the beat. Transfer the heart back to the frog Ringer's solution, and stimulate it with a mild electrical shock. Observe and interpret the results.

If the heart fails to respond, inject a few drops of adrenalin (1:10,000 parts) into the ventricle. If this fails, prick the heart with a pin to restore heart beat.

(1) *Advance an hypothesis to explain the effect of ions on the heart cells involved.*

Mechanical and electrical stimulation Remove the visceral organs to expose the posterior spinal nerves. Pinch some of these nerves with forceps. Observe and record the results.

Apply a very mild electrical stimulus by means of copper wire attached to a dry cell. Observe and record the results.

(2) *Does a continuous unvarying electrical stimulus applied to a nerve produce a continuous unvarying response? Explain.*

Nervous Coordination - Receptors

OBJECT *to locate some types of sensory receptors.*

MATERIALS

Applicator sticks—16	Absorbent cotton
Sharp pin	Beaker of iced water
Beaker of hot water	Four-inch nails—6
Wax marking pencil	Dissecting scissors—1 pair
Clean tap water—four 400 ml. beakers full for rinsing mouth	
5% sucrose solution—10 ml.	
10% NaCl solution—10 ml.	
1% acetic acid solution—10 ml.	
Aspirin solution (1 tablet in 10 ml. water)	
or 0.001% quinine sulphate solution—10 ml.	
Beaker of 75% ethanol in water for sterilizing	

PROCEDURE

Work in pairs — one student acting as the subject while the other performs the tests and records the results. Alternate the functions, and repeat all the tests. The subject must keep his eyes closed during the tests or wear a blindfold.

Temperature discrimination Place 3 four-inch nails in iced water and 3 in hot water. With a wax pencil draw a one-inch square on the palm and another on the back of the hand. Subdivide each square by grid lines making 16 smaller squares. Draw two similar but larger grids in your notebook for recording the results.

While the subject's hand rests on the desk, explore the marked skin areas for cold and heat receptors. To do this, lightly apply the tip of an iced nail or a hot nail to one of the squares of the grid on the skin. Use a random selection of hot and cold nails for the tests, and record the results as positive if the subject identifies the type correctly, or negative if the response is incorrect. All grid squares should be tested for both hot and cold receptors. Use a blue pencil for recording cold responses and a red pencil for heat responses.

Pain discrimination The same skin grids used for temperature tests should be used for this exercise. Draw new recording grids in your note-

book. Using a sterilized pin, test the marked skin areas for pain-receptor distribution. Follow the same testing and recording procedure as used above for temperature discrimination. The subject must distinguish carefully between touch or pressure and pain. Use light pricks, being careful not to plunge the pin too deeply into the skin. Allow several seconds between pin pricks to permit previous sensations to wear off.

Taste discrimination Roll a small swab of absorbent cotton on one end of an applicator stick. The subject must rinse his mouth before and after each series of tests. Dip one of the applicators into a 5% sucrose solution, and touch the moistened cotton to the following regions of the tongue: tip, front side edge, upper front centre, upper back centre, back side edge, and lower centre. Record where a sweet sensation is most distinct, less distinct, or not registered at all.

Using separate applicators, repeat the above procedure with each of the following solutions in order: 10% sodium chloride, 1% acetic acid, aspirin or quinine solution. All tests must be carried out by each member of the group. Record all data.

Make an outline sketch of the human tongue. Shade and label the areas sensitive to the following taste sensations: sweetness, sourness, saltiness, and bitterness.

Blind spot Using a wax marking pencil, draw a heavy circle about $\frac{1}{2}$ inch in diameter on a sheet of white paper. About 4 inches to the right of the circle, draw a heavy cross about the same size as the circle. Cover the left eye with one hand and hold the sheet of paper about 20 inches away from your face. Direct your gaze on the circle and slowly move the paper nearer. At a certain distance the cross will disappear from view but will reappear as the sheet is brought closer.

- (1) *Explain why the cross disappeared from view and then reappeared as the paper was brought closer.*
- (2) *What are the relative numbers of cold, heat, and pain receptors?*
- (3) *Discuss the structure of different types of cutaneous receptors.*
- (4) *What other neural receptors not dealt with in this exercise does man possess?*

Reproduction - Sexual

OBJECT *to study reproduction in Drosophila melanogaster Mg. (fruit fly).*

MATERIALS

Incubator	Medicine dropper
Refrigerator	Paper towel
Yeast-glucose suspension	Ether
<i>Drosophila</i> culture bottles (see appendix)	
Stock culture of <i>Drosophila</i> (wild type)	
<i>Drosophila</i> Guide—Demerec and Kaufmann (see index)	

PROCEDURE

Anesthetize two or three female flies and one or more males (see appendix). Put them in a small cup or cone of paper and lower them into a culture bottle which has a piece of dry paper towelling in the bottom. Try to place the flies on the paper towelling. Anesthetized flies often stick to a damp medium and are unable to free themselves. Moisten the culture medium each day with yeast suspension to feed the flies. Do not flood. One-third of the class should incubate their cultures at 25°C. (NOT OVER 27°C.), one-third at room temperature, and one-third in a refrigerator or other cool location. Mark culture bottles with the date, incubation temperature, and your name.

Your records should show: *Date of beginning incubation, incubation temperature, length of time required for larvae to appear, for pupae to appear, for adult flies to emerge, and the average number of offspring per female parent.*

When adult emergence is complete, saturate the plugging cotton with ether and cover the culture bottle with an inverted beaker to kill all flies in it. Turn the dead flies out onto a sheet of white paper and count the numbers of male and female flies. (See *Drosophila* Guide, Demerec and Kaufmann, page 8).

(1) *Did incubation temperature make any significant difference in the time required for the flies to complete their life cycles?*

- (2) *Compare the numbers of male and female offspring.*
- (3) *How is sex determined and how is it inherited? How closely does your experiment substantiate your explanation?*
- (4) *Outline the life cycle of *Drosophila melanogaster* indicating where, in the cycle, meiosis, mitosis, and fertilization occur.*

Probability and Heredity

OBJECT *to demonstrate the role of chance in inheritance.*

MATERIALS

Dime
Cent

PROCEDURE

In speaking of the chance of something happening, mathematicians use the term probability and represent this by the symbol p . If something is certain to happen, p has the value 1; if it is certain not to happen, the value of p is 0. The probability of throwing a six with a dice is $1/6$. If, as in tossing a coin or holding the two parts of a wishbone, one in either hand, two alternatives (heads or tails, the long or the short part in the right hand) are equally likely; each has the probability 0.5. The probabilities of two alternatives are usually expressed as p and q , so that $p + q = 1$. But, you will realize that 10 throws of a coin do not always give five heads and five tails.

In the formation of germ cells by meiosis, the same situation prevails as with coin-tossing. The probability that any sperm (or any egg) produced by an Aa individual will carry the A allele is 0.5; the same is true for a .

The dime represents the egg, the cent represents the sperm, regard heads as A and tails as a in both coins. Toss the coins simultaneously 100 times and record the results.

- (1) *Estimate the probabilities of A and a from your 100 female chances, from the 100 male chances, and from the total 200 chances.*
- (2) *Estimate p for A from the entire class results, and from the results accumulated since this exercise was first done in your school.*

The results of tosses of single coins can be regarded as populations of germ cells, those of simultaneous tosses of pairs of coins as offspring of the next generation by fertilization of the ova by the sperm.

- (3) *What is the relation between population size and agreement with the theoretical (1:1) ratio? Explain.*

(4) *Expand the binomial $(pA + qa)^2$ where $p = q = 0.5$.*

The theoretical ratio of $AA:Aa:aa$ when applied to 2 coin combinations, and also when applied to the genotypic ratio in the offspring of heterozygous parents of genotype Aa , can be found from the expansion in (4). In this case $AA:Aa:aa = 1:2:1$.

(5) *Compare the results obtained in your simultaneous coin tosses with those expected from (4).*

(6) *If, as most frequently happens, A is dominant to a , the genotypes AA and Aa will be indistinguishable. What will be the theoretical ratio of the combination of these two genotypes to aa in (4), and what will be the actual ratio from your coin tosses as derived from (5)?*

Heredity - Albinism

OBJECT *to study albinism in sorghum.*

MATERIALS

100 grains of albino sorghum (Kaffir corn)
Seed flat (12" x 12" x 3")
Loam to fill seed flat

PROCEDURE

Plant 100 grains of sorghum in moist soil in the flat. Set in a warm, light location. Keep the soil moist and examine periodically. In 8-12 days the seedlings will have been developed sufficiently to permit green and albino plants to be distinguished. Count and record the numbers of each. Consolidate the results of the class.

- (1) *Is environment a factor in causing albinism? Explain.*
- (2) *Albinism in sorghum is lethal. Why?*
- (3) *What further experiments could be done to determine the percentage of the green plants which are hybrids?*
- (4) *In view of the fact that albinism is lethal in sorghum, explain how seeds for albino sorghum are produced.*

Heredity - Pure and Hybrid Strains

OBJECT *to study the transmission of an hereditary trait from generation to generation in *Drosophila melanogaster* Mg.*

MATERIALS (per group of two students)

Pure stock culture of *D. melanogaster*—wild
Pure stock culture of *D. melanogaster*—vestigial wing
Prepared *Drosophila* culture bottles—2 (see appendix)
Yeast-glucose suspension—2 ml.
Medicine dropper
Etherizer (see appendix)
Re-etherizer (see appendix)
Ethyl ether—10 ml. in tightly capped bottle
Drosophila Guide—Demerec and Kaufmann (see appendix)

PROCEDURE

Work in pairs.

F₁ generation Obtain one wild type male fly and two or three virgin vestigial-wing females from those etherized by your instructor. Place the etherized flies in a small paper cone and put this into a prepared culture bottle held on its side. Replace the cap or cotton plug of the culture bottle. Label the bottle with the date, type and sex of the flies, and your name. Set aside at room temperature.

After eight or nine days remove the parent (P_1) flies. About the tenth to the twelfth day the offspring (F_1) should begin to emerge. Remove them each day and transfer them to a killing bottle. Remove the dead flies daily from the killing bottle, examine them carefully, and make an accurate record of their wing type.

After about three days, instead of killing all the young flies, select two or three females and one or two males and transfer them to a fresh culture bottle. This mating of the F_1 offspring (P_2) is to be used for the next part of this exercise (F_2 generation).

F₂ generation After the F_1 s have been in the culture bottle for eight or

nine days, remove and kill the adult flies. Each day as the F_2 generation starts to emerge, transfer them to the killing bottle. Keep accurate daily records of the wing types which appear over the next five to seven days.

- (1) *From your observation of the F_1 generation, which trait, normal or vestigial wing type, is dominant?*
- (2) *From your records of the F_1 generation, determine the phenotypic ratio of normal wing to vestigial wing.*
- (3) *Represent this cross by a diagram, giving the genotypes and phenotypes of the parents (P_1) and F_1 s.*
- (4) *Derive the genotypes and their theoretical proportions for the F_1 progeny.*
- (5) *State your conclusions regarding the inheritance of these traits.*
- (6) *How would you define a gene on the basis of the F_1 and F_2 results?*

Appendix - Solutions and Laboratory Procedures

Quantities of stock solutions are for a class of 30 students

Amphibian Ringer's Solution (For frog tissue) Dissolve the following compounds in 1 liter of distilled water: NaCl — 6.50 g., NaHCO₃ — 0.20 g., KCl — 0.14 g., CaCl₂ — 0.12 g.

Blood — Whole Possible sources of supply: slaughter houses, hospitals or Red Cross — outdated blood, freshly killed animals.

NOTE: If blood is to be collected fresh, an anticoagulant should be added immediately to prevent clotting. To prepare the anticoagulant, dissolve 2 grams of potassium oxalate and 6 grams of sodium chloride in 100 ml. of distilled water. For every 10 ml. of fresh blood add 1 ml. of anticoagulant. Blood can be defibrinated by whipping it with a glass rod.

Brom Thymol Blue Stock Solution Dissolve 40 mg. of powdered brom thymol blue dye in 3 ml. of 95% ethanol. Add this to 96 ml. of distilled water.

Carbon Dioxide Generator Put about 25 grams of marble (calcium carbonate) chips in a 300 ml. florence flask. When ready to use add 100 ml. of cold 50% H₂SO₄. (CAUTION: *Add the acid to the water.*) Stopper the flask with a one-hole rubber stopper to which a glass delivery tube has been fitted.

Chlorophyll Stock Solution Kill 50 grams of green leaves by placing them in boiling water for a minute or two; this makes the cell membranes permeable to alcohol. Place the dead leaves in a 400 ml. beaker and cover with 200 ml. of 95% ethyl alcohol. Heat in a water bath until the leaves are devoid of all colour. (CAUTION: *Alcohol is highly inflammable.*)

Pour the alcohol containing the pigments into a labelled bottle and store in a dark place.

Drosophila Culture Medium and Preparation of Culture Bottles (see Lab. 29) The following recipe is suggested (about 20 portions): 20 g. agar-agar; 1000 ml. water; 100 g. cornmeal (yellow); 70 ml. Karo corn syrup; 70 ml. dark molasses; 2 g. sodium propionate (mold inhibitor) dissolved in 5 ml. of 95% ethanol.

First add the agar-agar to the water and bring to the boil. The agar should dissolve, producing a smooth liquid. Add alcoholic solution of mold inhibitor and slowly add the corn meal, stirring continuously to prevent lumping. When it is well mixed, add both syrup and molasses and allow to boil slowly for ten minutes. If cooked too long the medium will harden in the dish. Remove the medium from the heat and pour it at once into sterilized $\frac{1}{2}$ pint milk bottles; about one-half inch depth of medium is enough. Be careful not to smear the medium on the inside of the bottle. Use a funnel if necessary.

When the medium has been poured into the bottles, strips of paper towelling cut to the length of the bottle and folded double should be inserted into the medium so that the larvae will have a place on which they may crawl to pupate. Plug each bottle with a wad of non-absorbent cotton wrapped in a square of cheese cloth.

Sterilize the bottles in an autoclave or pressure cooker for about 15 minutes at 15 pounds per square inch. Do not use the bottles until they have cooled. Shortly before use, yeast must be added to provide food for the flies. Several grains of dry baker's yeast should be sprinkled on the solidified medium.

Normally the medium should be less than 48 hours old when used, but refrigeration or freezing will greatly extend the storage time.

Frog Pinning Board Frog pinning boards may be prepared by cutting out 4" x 6" pieces of insulating board, ceiling tiles, or wall board. The materials are available from building supply outlets. Use a cork borer to cut a hole $\frac{1}{2}$ " in diameter near one corner. (See fig. 9.)

Human Ringer's This solution, isotonic for mammalian tissues, may be used as mounting fluid for examination of living tissues. Prepare this solution in 1 liter of distilled water: KCl — 0.42 g.; NaCl — 9.0 g.; CaCl_2 — 0.24 g.; NaHCO_3 — 0.20.

Incubator (Water bath) An improvised water bath may be prepared by putting about 4 inches of water in a container such as an aquarium, or a metal tank. Heat the water with an aquarium heater controlled by an aquarium thermostat set at the proper temperature. If materials in test tubes are to be incubated, test tube racks should be placed in the water to prevent the tubes from upsetting. This water bath should be set up well in advance of when it is needed so that the temperature can be adjusted and can stabilize. Incubation at body temperature is conveniently carried out in closed tubes in the pants pocket.

Iodine — Potassium Iodide Solution (I, KI) Dissolve 3 grams of potassium iodide in 25 ml. of distilled water and then add 0.6 g. of iodine crystals. Stir until dissolved. Make up to 200 ml. with distilled water.

Lime Water Solution To make lime water, add an excess of calcium hydroxide (about 10 grams) or calcium oxide to 500 ml. of distilled water. Stopper and shake thoroughly. Let stand for twenty-four hours and decant the supernatant fluid into a labelled bottle. Filter if necessary to obtain a clear solution.

Lugol's Solution Dissolve 10 grams of potassium iodide in 100 ml. of distilled water. To this add and dissolve 5 grams of iodine crystals. Store in a labelled stock bottle.

Gram's iodine may be prepared from this solution by diluting with fourteen volumes of distilled water.

Oxygen Generator Mix 1 gram of manganese dioxide with 10 grams of potassium chlorate. Place the mixture in an ignition tube. Stopper the

test tube with a one-hole rubber stopper to which a glass delivery tube has been fitted. Heat as required with a bunsen burner to produce oxygen.

Pancreatin Solution (Prepare fresh) Add 10 grams of powdered pancreatin to 90 ml. of distilled water. Refrigerate in a labelled bottle. Shake well before using.

Pepsin Solution (Prepare fresh) Add 10 grams of powdered pepsin to 90 ml. of distilled water. Refrigerate in a labelled bottle. Shake well before using.

pH Indicators:

Indicator	Colour change	pH range
Congo red	blue to red	3 to 5
Methyl orange	orange-red to yellow	3 to 4.4
Litmus	red to blue	4.5 to 8.4
Brom thymol blue	yellow to blue	6 to 7.6
Phenol red	yellow to red	6.6 to 8.2
Neutral red	red to yellow	6.8 to 8
Phenolphthalein	colourless to red	8.2 to 10

Potassium Pyrogallate Solution (Prepare fresh) Dissolve 2 grams of pyrogallic acid crystals and 10 grams of potassium hydroxide in 60 ml. of distilled water. Store in full, tightly-stoppered bottle until used.

Powdered Skim Milk Stock Solution (Prepare fresh) Dissolve 15 grams of powdered skim milk in 90 ml. of distilled water. Refrigerate until used.

Rennin (Junket) Stock Solution (Prepare fresh) Dissolve 3 rennin tablets in 22 ml. of distilled water. Refrigerate until used.

To Prepare Spinal and Pithed Frogs An excellent descriptive and pictorial presentation of these preparations is presented on pages 128 and 129 of Morholt, Brandwein, Joseph, *A Source Book for the Biological Sciences*, Harcourt, Brace and Company, Inc., New York, 1958.

Yeast-Glucose Suspension (Prepare fresh) Suspend 10 grams of dry baker's yeast in a solution of 10 grams of glucose in 90 ml. of distilled water. Refrigerate until required.

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FORMULAE; MATERIALS AND EQUIPMENT REQUIRED

	LAB. NO.		LAB. NO.
Acetic acid	22, 28	Benzene C_6H_6	11
Acetylcholine — 1 x 10 cc. vial per class of 30	24	Bicarbonate ions	4
Acrolein — odour of	16	Bile salts	16
Adhesive tape — $1\frac{1}{2}$ ", — 1 roll per 8 students	10	Binomial theory	30
Adrenalin sulphate — 1 x 10 cc. vial per class of 30	24, 27	Biuret	2
Adsorption — of particles	8	Blind spot	28
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Ammonium hydroxide NH_4OH	6	Brown paper — for fat test	3
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Balance — triple beam — 1 per 4 students		Carbon dioxide	
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Beakers — 1 set of 50 ml., 100 ml., 250 ml., 400 ml. per 2 students		in photosynthesis	13
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		guard	10
		Cellular excitation	27
		Cent	30
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		Cerebrum — of frog	26

	LAB. NO.
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Chlorophyll stock solution	10 app.
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in frog capillary	24
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	LAB. NO.
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	LAB. No.		LAB. No.
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Fructose	1	Intestine — small	16
Fruit fly	29, 32 & app.	Iodides	4
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	LAB. NO.
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Mercuric chloride $HgCl_2$	2
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Monosaccharides	1
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Olive oil — 4 ozs. per class of 30	3, 9, 16
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Osmosis	7
Ovum	30
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Oxygen	
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Petri dish — 1 per 4 students	13
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pH	
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	LAB. NO.
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Pinning board — for frog	24 & app.
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Population — of fruit fly	30
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Potassium	
chlorate $KClO_3$	22
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oxalate	app.
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Rubber hose, $\frac{1}{8}$ " — 3' per 4 students	22
Rubber hose, $\frac{1}{8}$ " — 2 $\frac{1}{2}$ ' per 4 students	22
Safranin dye — 1% aqueous solution	10
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Sap — rise of	10
Scalpel — surgical — 1 per 4 students	
Scalpel blades — 1 per student	
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Seeds			
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Slides — plain for microscope — 1 per student			
Slides — prepared — of human blood	22		
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Stirring rods, 8" — 2 per 4 students			
Stomates	10		
Stoppers			
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solid, gum rubber —			
#2 — 13 to 20 mm. —			
2 lbs. per class of 30			
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3 lbs. per class of 30			
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2 lbs. per class of 30			
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2 lbs. per class of 30			
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carbon dioxide	17		
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oxygen	12		
protein	1		
unknown	4		
Test tubes			
#4 — 15 x 125 mm. —			
1 doz. per 4 students			
#6 — 18 x 155 mm. —			
1 doz. per 4 students			
#17 — 25 x 300 mm. —			
1 doz. per 6 students			
18 x 100 mm. — no lip	18, 19		
Test tube brush — 1 per 4 students			
Test tube holder — 1 per 2 students			
Test tube rack — 1 per 2 students			
Thread — 1 spool #40 per class of 30	27		
Tonicity	7		
Tongs — 1 pair per 4 students			
Tongue — taste sensations	28		
Tooth picks — 1 box per class of 30	23		
Transpiration	10		
Transport of water in plants	10		
Urine	5		
Vacuum wax — 1 lb. per class of 30	18		
Vascular bundles — in stems and petioles	10		
Vaseline — 1 jar per class of 30	10		
Ventilation — forced	17		
Viable seeds — 50 ml. of any one of the following:			
wheat, oats, radish, barley, etc.	18		
Vials — glass with screw cap — 21 x 70 mm. — 1 per 4 students			
Wandering Jew plant (<i>Zebrina</i>) — 1 plant per class	10		
Water bath — see incubator			
Water glass — see sodium silicate	7		
Watch with second hand — 1 per 4 students	24, 25, 27		
Water transport in plants	10		
Wax marking pencil — 1 per student	8, 19		
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Wire asbestos gauze — 1 per 4 students	12, 14		
Wire — copper — insulated — 10 to gauge — 4 ft. per 4 students	22		
Xanthophyll	11		
Xylem — conduction in	10		
Yeast			
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<i>Zebrina</i>	10		

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